A contact zone between two distinct *Rana temporaria* lineages in northern Germany

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Recolonization of northern Europe after last glaciations has left distinct marks on its biota, and several contact zones between different refugial-lineages have been identified. Earlier studies of the common frog (*Rana temporaria*) have revealed two old (c. 0.75 Myr) mitochondrial DNA (mtDNA) lineages occurring in Europe, but the location of the contact zone has not been identified. Here we have performed mtDNA haplotyping for individuals from five populations form the suspected contact zone area in northern Germany. The distributions of the eastern and western mtDNA haplotypes strongly support a secondary contact zone between these two lineages, situated in between the rivers Weser and Elbe. This finding contributes not only to our understanding of the biogeography of European fauna, but also offers an opportunity for studies of introgression across a relatively narrow contact zone.

INTRODUCTION

The genetic structure of populations is determined not only by current population dynamics, but also by historical patterns of species distribution and gene flow. Especially in the northern latitudes, the biotic communities have been strongly shaped by the intermittent advances of continental ice sheets in the Middle and Late Pleistocene. These events have repeatedly restricted the distributions of most of the species to the southern refugial regions – to the Iberian and Apenninean Peninsulae, Balkans and south-western Russia in Europe (HEWITT, 2000). In many cases, the migrational barriers between the refugia have given rise to distinct intraspecific genetic lineages (see e.g. HEWITT, 1996) and possibly accelerated the pace of speciation (e.g., WEIR & SCHLUTER, 2004; but see e.g. KLICKA & ZINK, 1997; ZINK et al., 2004). During the interglacials of the Middle and Late Pleistocene, organisms surviving in the refugia rapidly recolonized the previously glaciated, now free habitats. As the colonization
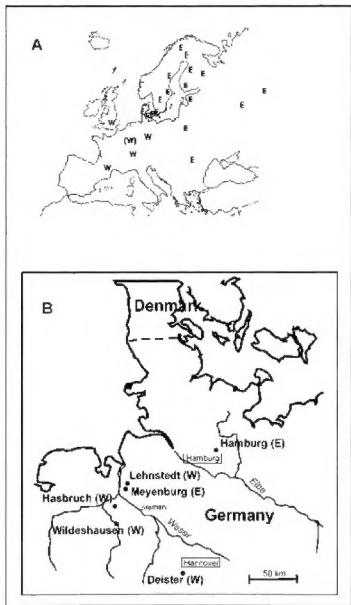
Fig. 1. – (A) The distribution of western and eastern Cyt*b* haplotypes in the European *R. temporaria* populations following results from VEITH et al. (2003) and PALO et al. (2004*b*). The two lineages are indicated by W for the western lineage and E for the eastern lineage. The population from Koblenz, as analysed by VEITH et al. (2003), is marked between brackets. (B) The sample locations (black dots) in the presumed contact zone. The Deister population was analysed in PALO et al. (2004*b*). The grey areas denote major cities.

Tab. 1. – The study populations, their coordinates and the numbers of observed western (n_w) and eastern (n_e) Cyt$b$-haplotypes in each of the populations.

Population	Approximate coordinates	n_w	n_e
Wildeshausen	52°54'07''N, 08°25'34''E	1	–
Hasbruch	53°05'42''N, 08°25'17''E	4	–
Meyenburg	53°16'39''N, 08°35'52''E	5	48
Lehnstedt	53°19'20''N, 08°38'34''E	15	–
Hamburg	53°40'51''N, 10°11'19''E	–	3

proceeded, the different refugial lineages met, forming narrow contact zones. In Europe, major contact zones have been identified in Fennoscandia, central Germany, and in the mountain ranges of the Pyrenees and Alps (summarized in e.g. TABERLET et al., 1998; HEWITT, 1999).

Rana temporaria Linnaeus, 1758, the common frog, is a middle-sized brown frog with wide distribution in Europe (GASC et al., 1997). A recent assessment of Cytochrome b (Cyt$b$) gene sequence variation in *R. temporaria* identified two old (c. 0.75 Myr) mitochondrial DNA (mtDNA) lineages occurring in western and eastern/northern Europe, respectively (PALO et al., 2004b). Unlike in many other terrestrial organisms, the Fennoscandian populations harboured exclusively the eastern mtDNA haplotypes, whereas individuals sampled from Spain, Switzerland, Great Britain and Germany (fig. 1) carried the western mtDNA haplotypes. The study, however, failed to identify the exact location of the contact zone, but suggested Danish mainland/northern Germany as a likely area. This was based on the apparent occurrence of nuclear microsatellite variation typical for the western/central European populations observed in the Danish populations, which yet carry the eastern mtDNA type. In this study, we extended the previous mtDNA results based on more detailed sampling of the northern German populations to shed light on the location of the secondary contact zone.

MATERIAL AND METHODS

Seventy-five *R. temporaria* individuals from five locations in northern Germany (tab. 1) were screened for the presence of eastern or western haplotype (as defined in PALO et al., 2004b) in 605 bp of Cyt$b$ sequence. The adult toe-clip samples (locations Wildeshausen, Hasbruch) were collected during the migration towards the breeding pond in spring 2004. The samples from the Hamburg area stem from the collection of the Zoological Museum in Hamburg (voucher numbers: A.03155, A.03505, A.04438). The Meyenburg and Lehnstedt were independent tadpole samples, collected during summer 2004.

DNA was extracted using the method suggested by ELPHINSTONE et al. (2003). The two phylogenetic lineages were distinguished by restriction fragment length polymorphism

(RFLP) method, based on the presence/absence of a diagnostic *Sty*I restriction enzyme cut site at PCR amplified partial Cyt*b* gene sequences. For information about the primers, PCR and RFLP conditions, see PALO et al. (2004*b*). To check for potential non-informative point mutations affecting the enzyme cut site, five western mtDNA haplotypes (GenBank accession number: EF507844) identified with the RFLP method were sequenced as described in PALO et al. (2004*b*). We also note that an incorrect species identification (e.g. between morphologically similar *R. arvalis* and *R. temporaria*) can create an erroneous Cyt*b* signal with the present RFLP method. Therefore, the species identification of all the individuals was confirmed with the method suggested by PALO & MERILÄ (2003).

RESULTS

The six *R. temporaria* individuals from Hasbruch and Wildeshausen, west of river Weser (fig. 1), harboured the western mtDNA type, whereas the majority of Meyenburg and the three Hamburg samples (*n* = 56), east of the Weser, carried the eastern type (tab. 1). Interestingly, the 15 samples from Lehnstedt, east of river Weser and only c. 6 km away from the Meyenburg sampling location, possessed exclusively the western type mtDNA (tab. 1; fig. 1).

DISCUSSION

Our RFLP results support the existence of a contact zone between the western and eastern *R. temporaria* lineages in northern Germany between the rivers Weser and Elbe. Unfortunately, the current sampling scheme does not allow the localization of the contact zone further down to the South. Yet, the occurrence of western type Cyt*b* for two populations from the vicinity of the cities Hannover and Koblenz has been previously confirmed (VEITH et al., 2003; PALO et al., 2004*b*).

For a number of terrestrial and aquatic species, the Fennoscandian region was colonized postglacially by two or more distinct refugial lineages (e.g., TABERLET et al., 1995; JAAROLA et al., 1999; KONTULA & VÄINÖLÄ, 2001; FERRIS et al., 1998). Contact zones between the intraspecific lineages appear to cluster in central Sweden, around the area where the ice persisted the longest after the last glaciation (TABERLET et al., 1998). *R. temporaria* populations, harbouring exclusively the eastern mtDNA lineage in northern and eastern Europe, do not fit to this general pattern. Although the colonization of Fennoscandia solely by one lineage is atypical, a central European contact zone is known from a number of other species as well (HEWITT, 2004).

The current distribution *R. temporaria* mtDNA diversity in the North of Europe resembles geographically the diversity of the eastern and western mtDNA lineages observed in the fish *Leuciscus cephalus* (DURAND et al., 1999). It was shown that the Fennoscandian *Leuciscus* populations harbour exclusively the eastern mitochondrial type. The secondary contact zone is located in northern Germany, where a population from the Elbe has the eastern type, and a population from the Weser has the western type mtDNA. From this area,

the distribution of the western mtDNA extends to the Southwest as well as up to the Balkans in the Southeast along the Carpathian mountain range. A contact zone between the rivers Elbe and Weser have also been found e.g. in the butterfly *Polyommatus coridon* (SCHMITT & SEITZ, 2001) and in the moor frog *R. arvalis* (BABIK et al., 2004). Contact zones of other species are also reported to occur further south or south-west, along the French-German border region (e.g., TABERLET et al., 1998) or in central Germany (MÜLLER, 1998).

An interesting feature of the results in this study was the exclusive presence of eastern and western mtDNA haplotypes in the two populations separated only by c 6 km. This fits to the general picture that the gene flow over contact zones is very limited (IBRAHIM et al., 1996, HEWITT, 1999). Nevertheless, in *R. temporaria* there is significant mixing of nuclear alleles between the western and eastern lineages over a rather broad geographical area in Denmark (PALO et al. 2004b). The clearcut differences of female-mediated mtDNA diversity at the contact zone are especially striking given the fact that migration rates in *R. temporaria* are female-biased (PALO et al., 2004a). Whatever the explanation, the results of this study have narrowed down the location of a secondary contact zone between two divergent lineages of frogs and thereby pinpoint the relevant scale and area where the hybrid-zone is likely to be found.

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